THE PROCESS OF EGG-MAKING IN A TREMATODE.

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An outline of the process here described was published by me in connection with the description of the species *Epibdella bumpusii*, an ecto-parasite of the sting ray (*Dasyatis centrura*).[1]

Since opportunities of witnessing this instructive phenomenon are rare, and descriptions are wanting, even in the larger text-books, and, further, since the knowledge of the process has proved to be of great value in explaining to students the anatomy of the reproductive apparatus of the trematodes, I venture to publish a description of the process as I have seen it.

Every teacher of zoölogy is familiar with the anatomy of some typical trematode. The description of the relations of the various parts of the complicated reproductive machinery is about as far as one is apt to go in lecturing to a class, unless he has happened to see the machinery in operation, or, possibly, has had access to a description of the physiological working of that machinery. If what I have here written will help to vivify now and then a lecture on the reproductive apparatus of the trematodes I shall be repaid for the small trouble it has been to write out this narrative.

The species *E. bumpusii* is peculiarly well adapted for the study of the process of egg-making. The animal is small enough to allow of satisfactory study with low magnification, and is, withal, transparent enough to permit rather minute details to be clearly seen. Furthermore, the animal can be kept for several hours in sea water under slight pressure without impeding the normal action of the reproductive organs.

ANATOMY.

In this description only the anatomy of the reproductive organs is considered. Reference to the figures and the explanation of the same will be necessary in order to follow this description.

As in most trematodes the individuals in this species are her-

[1] Bulletin U. S. Fish Commission for 1899, pp. 286-7, figs. 11-15.

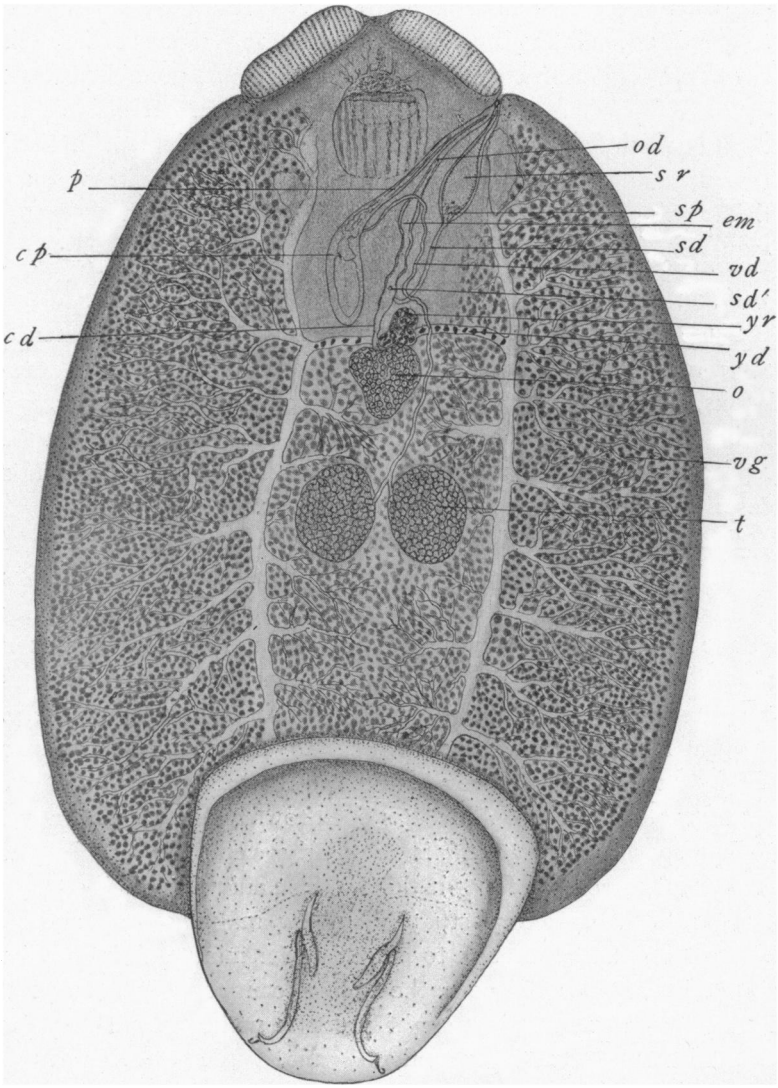


FIG. 1. *Epibdella bumpusii* Linton, ventral view; vitellaria partly diagrammatic. Actual diameter at level of testes 4.5 mm.

maphroditic. The male genitalia consist of an eversible cirrus (*p*), a cirrus-pouch (*cp*), in which is the seminal vesicle (*sv*). A vas-deferens (*vd*), which follows a somewhat tortuous course, may be easily traced from the seminal vesicle to the two testes

(*t*), which are subglobular in shape and lie near each other transversely placed and a little in front of the middle of the body. The cirrus pouch pulsated rhythmically and continued to pulsate long after all movement had ceased in the other organs.

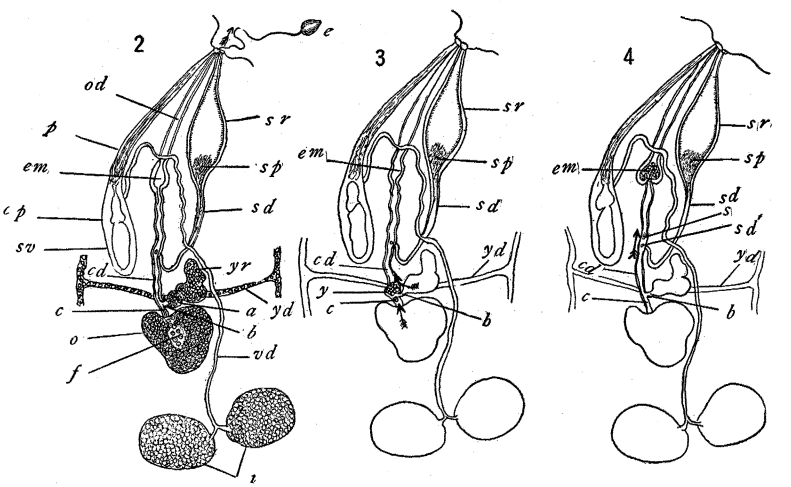
The female genitalia are more complicated. Filling the body laterally and posteriorly are the vitellaria (*vg*). These are dendritic glands whose ducts ultimately are collected into a transverse duct (*yd*) which enlarges into a vitelline reservoir (*yr*), lying a little to the left and a little in advance of the germarium (*o*). Immediately in front of the testes is the germarium (*o*), a somewhat trilobed organ lying on the median line. A short duct (*c*) passes at first dorsad then cephalad from the germarium where it joins another short duct (*ó*) coming from the vitelline reservoir (*yr*). The common duct (*cd*) then passes near the median line, cephalad to the shell mold (oötype) (*em*), which lies near the cirrus-pouch and to its left. About midway between the germarium and the oötype the common duct is joined by a minute duct (*sd*, *sd'*), which enters on its dorsal side and comes from the seminal receptacle (*sr*). The seminal receptacle lies to the left and a little in advance of the oötype.

The cells which constitute the shell-forming gland surround the common duct for some distance back of the oötype. The oviduct passes forward from the oötype, lies beside the cirrus, and opens with it in the genital notch which lies on the anterior border on the left side of the head. The vagina opens in the genital notch close to the left side of the oviduct. It leads into a capacious seminal receptacle (*sr*), from the caudal end of which the seminal duct (*sd*), above mentioned, proceeds mediad to the common duct from the vitelline reservoir and germarium.

Physiological Action of the Genitalia which are Concerned in the Process of Egg-Making.

Following are the events named in order as they were seen to occur in a specimen which was lying in sea water under a coverglass. First, a lobe (*a*) of the vitelline reservoir (*yr*) contracted vigorously thereby emptying itself of a definite mass of the coarsely granular vitelline substance with which it was filled. As this takes place in a definite portion of the reservoir, it fol-

lows that approximately the same amount of yolk is thus discharged at each contraction. The mass of yolk is propelled with comparative rapidity along the short vitelline duct (*b*) towards the median line. This duct passes near the anterior border of the germarium (*o*), where it is joined by another short duct (*c*) from the germarium. As the mass of yolk was passing the germarium it was noticed that a number of free nucleated cells, which appeared to be lying loosely in a median area (*f*) of the germarium, were set in oscillatory motion. It was quite evident



FIGS. 2, 3, 4. Diagrams showing different stages of egg-making.

FIG. 2. An egg (*e*) has just been discharged from the oviduct (*od*). A mass of yolk for the next egg is collecting in a muscular lobe (*a*) of the yolk reservoir and is about to be ejected through the short duct (*b*) into the common duct (*cd*).

FIG. 3. The mass of yolk (*y*) has entered the common duct (*cd*) and its suction is probably an inciting cause of a germ's entering the common duct from the germ-duct (*a*). The direction of movement of yolk and germ is shown by the arrows.

FIG. 4. The mass of yolk has passed by way of the common duct to the oötype (*em*) where it has been molded into a tetrahedral shape and a shell has been deposited around it. A cluster of spermatozoa (*s*) has been injected into the common duct (*cd*) from the muscular seminal duct (*sd'*) at its opening (*sd''*) and has proceeded a short way towards the oötype. The arrow shows the direction of movement of the spermatozoa. In the process of egg-making the egg is discharged from the oötype a very short time (one or two seconds) after the spermatozoa have arrived at the oötype.

that the agitation of the germ-cells was due to the passage of the mass of yolk from the vitelline reservoir. While it was

certain, from what could be made out from the mechanism of the living worm, that one result of the rush of yolk in passing the germarium (see arrow in Fig. 3 lying partly on the yolk reservoir), was to draw a germ-cell from the duct (*c*) (see arrow on germarium, Fig. 3), no cell was seen to leave its place in the germarium to enter the common duct (*cd*). The reason for this failure to see a germ-cell join the mass of yolk appeared when sections were studied. It was then seen (Fig. 5) that the germ-duct (*c*) leaves the germarium on its dorsal side, and is therefore seen only in end view when the worm is flattened out on the slide. In so much of the duct as was visible there were many free germ-cells, all of them oscillating more or less and thrown into vigorous agitation at the time when a mass of yolk was

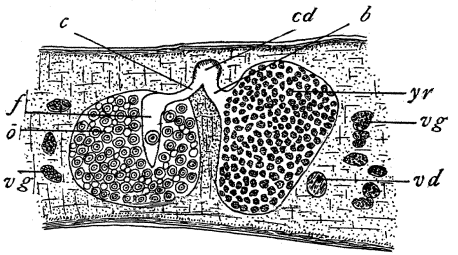


FIG. 5. Transverse section showing junction of ducts from yolk reservoir and germarium in common duct. Actual thickness of body through germarium 0.5 mm.

passing the germarium. If a cell were to leave the germarium its motion would be in a direction perpendicular to the eye, and therefore could not be detected. The portion of the duct which proceeds cephalad is somewhat concealed by the germarium. Hence here is one point in the process of egg-making, which, although perfectly obvious from the construction of the mechanism, was not actually seen. As the duct is at first perpendicular to the surface of the worm, it is obvious that the progressive motion of the germ-cell cannot be detected whether viewed from the dorsal or the ventral side.

The mass of coarsely granular yolk (Fig. 3, *y*) passes without pause and with comparative rapidity along the common duct (*cd*) to the oötype (*em*), which is simply a specialized part of the common duct. As soon as the mass of yolk reaches the oötype the passage closes by the approximation of the walls of the duct

thus forming a solid base on which the yolk rests. Against this base the mass of yolk is hammered by the walls of the oötype. Under this hammering the mass assumes a tetrahedral shape, and during the process the shell is built around it. The material for this shell is secreted by the gland which lies on each side of the common duct between the germarium and the oötype. When the shell is nearly finished, as shown by the cessation of the hammering process of the walls of the oötype, a very small and finely granular mass (Fig. 4, *s*) makes its appearance suddenly in the common duct at a point (*sd'*) approximately half way between the germarium and the oötype. This cluster of granules, which was in no case seen until it made its appearance in the common duct, travels rapidly along the common duct to the oötype. As soon as it reaches the oötype which now contains the newly encapsuled yolk mass with its associated germ-cell, there is a pause in the movements of the walls of the oötype and common duct for an instant. This pause is followed by powerful contractions of the walls of the oötype whereby the egg is forcibly ejected through the uterus (*od*) into the water.

The minute granular cluster which was seen entering the common duct immediately before the discharge of an egg, was interpreted at the time of observation to be spermatozoa, although the duct leading from the seminal receptacle to the common duct could not be seen in the living worm. It can be seen, however, in a stained specimen mounted in balsam and was satisfactorily demonstrated by means of serial sections.

Both the yolk mass and the spermatozoa appear to be propelled along the common duct by ciliary action. The egg is ejected by powerful muscular action of the walls of the oötype.

An examination of serial sections showed an interesting feature in the structure of the duct which leads from the germarium to the common duct. It is spacious at its beginning in the gland (*f*), where it appears in life as a clear space in the center of the germarium in which ripe germs could be seen oscillating whenever a charge of yolk was passing towards the oötype. The duct grows narrower distally. Indeed the duct (*c*) is shaped like a funnel. Near the point of union with the yolk duct (*b*) it is but little wider than the diameter of a single germ cell. A study of

sections makes it quite clear that the germ duct (*c*) and the yolk duct (*b*) connect in such manner that when a mass of yolk rushes along the yolk duct past the opening of the germ duct and into the common duct (Fig. 3, *b*, *c*, *y*, and arrow) sufficient suction is created to draw a germ from the germ duct.

Since the amount of yolk which is necessary for a single egg is automatically emptied into the yolk duct, the whole reflex has become adjusted with such marvellous nicety, the several parts to each other, that it seems probable that just enough suction is created to draw a single waiting germ cell from the germ duct which thus joins the passing charge of yolk.

The stimulus which causes a discharge from the seminal duct (*sd*, *sd'*) into the common duct always to take place immediately upon the completion of the process of molding the egg-shell is, of course, not obvious. The whole complicated process is an intricate nerve reflex.

Egg-making was observed to proceed actively for ten minutes or more to be followed by a short interval of rest. The time occupied in making an egg was not noted until the specimen had been under observation for two or three hours, by which time the worm had doubtless lost some of its vitality. When noted, the time from the moment at which the yolk left the reservoir until the completed egg was ejected into the water was about 40 seconds.

Summary of Events.

1. A mass of yolk leaves the yolk reservoir (*yr*).

2. As the yolk mass passes the germ duct (*c*) a germ is drawn out by the suction created by the moving mass of yolk.

3. The yolk mass and germ together pass along the common duct (*cd*) to the oötype (*em*).

4. An egg is molded into a tetrahedral shape by a kind of hammering action of the walls of the oötype, at the same time a shell is formed, its substance being secreted by the shell-forming gland.

5. A slowing up of the action of the oötype is followed by the appearance of a minute cluster of sperm in the common duct (*sd'*, *s*).

This cluster of sperms comes from the seminal duct and passes along the common duct to the oötype.

6. A momentary pause marks the arrival of the sperm at the oötype.

7. Powerful contractions of the walls of the oötype eject the egg from the uterus into the water.

Emendation of the Description of the Species.

In the original description of this species mention was made of but two pairs of hooks on the sucker. A third pair of minute hooks, posterior to the others, was subsequently found (Fig. 1).

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Explanation of Abbreviations.

Meaning of letters which are used in more than one figure.

b. Yolk duct leading from yolk reservoir to the common duct.
c. Germ duct leading from the germarium to the common duct.
cd. Common duct leading from the junction of *b* and *c* to oötype.
cp. Cirrus-pouch.
em. Oötype.
f. Beginning of germ duct in germarium.
o. Germarium.
od. Oviduct leading from oötype to exterior.
p. Cirrus.
sd. Seminal duct leading from seminal receptacle to common duct.
sd'. Point where *sd* enters the common duct.
sp. Spermatozoa in seminal receptacle.
sv. Pulsating organ at base of cirrus-pouch.
t. Testes.
vd. Vas deferens.
vg. Vitelline gland.
yd. Yolk duct leading from vitellaria to yolk reservoir.
yr. Yolk reservoir.